

# Improved Convergence in Dual-Topology Free Energy Calculations through Use of Harmonic Restraints

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**ABSTRACT:** When calculating free energy differences between two molecular systems by means of molecular dynamics simulation, accessory potential functions can help eliminate uninteresting configurational entropy contributions, improve convergence, and facilitate reversibility. In this work, we demonstrate that the use of a harmonic potential function to restrain key portions of a molecular system in a free energy perturbation dual-topology molecular dynamics approach dramatically improves convergence and precision of the calculation. Limitations of this technique are illustrated, and its use in conjunction with a fixed bond-length constraint is developed. © 1998 John Wiley & Sons, Inc. *J Comput Chem* 19: 1278–1283, 1998

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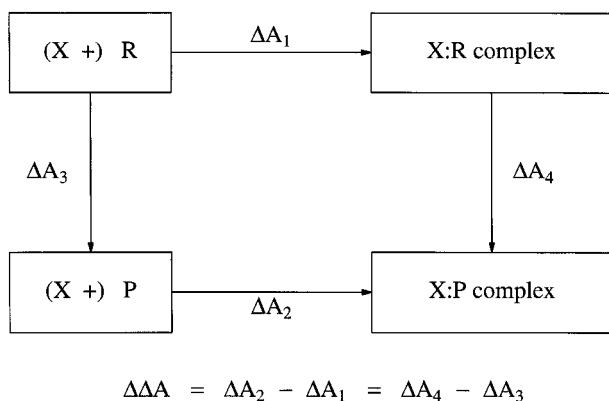
## Introduction

The free energy difference between two molecular systems may be calculated from molecular dynamics simulations using a thermodynamic cycle (Fig. 1) and either a single topological representation of the system or the so-called dual-topology approach.<sup>1</sup> Both approaches make use of a scalar reaction coordinate or coupling parameter,

$\lambda$ , representing the nonphysical conversion of one molecular species ( $\lambda = 0$ ) to another ( $\lambda = 1$ ). In single-topology approaches, the properties in a portion of the system are altered according to the value of the coupling parameter; in dual-topology approaches, the coupling parameter scales the relative contribution of two independent models to the overall energy function.

In practice, the two approaches have different strengths and pitfalls<sup>2</sup> and neither approach is entirely satisfactory. There are particular difficulties when one or more components of the system are annihilated in the course of the reaction. In the

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**FIGURE 1.** A basic thermodynamic cycle.

single-topology approach, they arise whenever an attempt is made to calculate a standard free energy of binding; in the dual-topology approach, they are always present at the extreme values of  $\lambda$ . These difficulties are illustrated by representing the potential energy,  $V$ , as

$$V_\lambda = V_X(\mathbf{r}_X) + (1 - \lambda)V_R(\mathbf{r}_X, \mathbf{r}_R) + \lambda V_P(\mathbf{r}_X, \mathbf{r}_P),$$

where  $R$  is the set of reactant atoms undergoing conversion to  $S$ ; the portion of the system that does not undergo conversion is  $X$ ; and  $\mathbf{r}_X$ ,  $\mathbf{r}_R$ , and  $\mathbf{r}_P$  represent coordinate vectors in configurational space for  $N_X$ ,  $N_R$ , and  $N_P$  atoms in corresponding portions of the system. The dependence of  $V_R$  and  $V_P$  on  $\mathbf{r}_X$  accounts for all interactions between these portions of the system and  $X$ . The free energy,  $A_\lambda$ , of this system is

$$A_\lambda = -k_B T \ln \frac{1}{S} \int e^{-\beta V_\lambda} d\mathbf{r}_X d\mathbf{r}_R d\mathbf{r}_P,$$

where  $\beta = (k_B T)^{-1}$  and the normalization factor  $S = S_X S_R S_P$ . Because  $S_X = \int \prod_{j=1}^{N_X} dx_j dy_j dz_j$  and likewise for  $S_R$  and  $S_P$ , we have  $S = v^{(N_X + N_R + N_P)}$  where the system volume  $v = \int dx dy dz$ . In the limit  $\lambda \rightarrow 0$ ,  $V_P$  becomes independent of  $\mathbf{r}_X$  (because there is no interaction between  $X$  and  $P$  when  $\lambda = 0$ ); it is then possible to separate the  $\mathbf{r}_P$ -dependent terms, yielding

$$\begin{aligned} \lim_{\lambda \rightarrow 0} A_\lambda &= -k_B T \lim_{\lambda \rightarrow 0} \ln \frac{1}{S} \int e^{-\beta V_\lambda} d\mathbf{r}_X d\mathbf{r}_R d\mathbf{r}_P \\ &= -k_B T \ln \frac{1}{S_X S_R} \int e^{-\beta(V_X + V_R)} d\mathbf{r}_X d\mathbf{r}_R \\ &\quad - k_B T \lim_{\lambda \rightarrow 0} \ln \frac{1}{S_P} \int e^{-\beta(\lambda V_P)} d\mathbf{r}_P. \end{aligned}$$

Thus, a fundamental problem with the dual-topology approach is that the final term on the right diverges as  $\lambda \rightarrow 0$ , rendering  $A_{\lambda=0}$  undefined. The same problem occurs as  $\lambda \rightarrow 1$ . The pseudophysical interpretation of this problem is that either the product or the reactant atoms become detached from the atoms to which they are bonded as  $\lambda \rightarrow 0$  or 1. In periodic boundaries, these atoms cannot leave the thermodynamic volume, and the problem reduces to one of adequately sampling this volume. In a typical stochastic boundary system, however, these atoms can leave the thermodynamic volume, thereby improperly contributing an unlimited amount of entropy because their configurational space is limited.

Thermodynamic cycle based approaches implicitly assume that these divergent terms cancel in the calculation of  $\Delta A_{0 \rightarrow 1} = A_1 - A_0$ . However, this can occur with precision only if all configurational space is thoroughly sampled, which is an unattainable ideal with present methods and resources.

Several recent efforts to calculate standard free energies of binding address this problem by means of positional restraints applied to portions of the system undergoing annihilation.<sup>3,4</sup> In some cases the restraints hold an atom within a specific region of space; in other cases they hold an atom within a molecule frame of reference. These restraints prevent nearly annihilated portions of a simulation system from escaping the regions of interest, but they also bias the system in a way that must be precisely removed to yield a final result.

The work reported herein represents a direct, but significant extension of this approach to the calculation of free energy differences using a dual-topology perturbation method. It differs from prior work in that the restraint is applied to portions of the system that would ordinarily not interact directly. This provides an analytical demonstration of its energetic contribution, automatic removal of the bias potential, and an extension to systems that employ SHAKE<sup>5</sup> constraints.

## Theory

Continuing with the symbols already defined, we introduce a harmonic potential that restrains the position of a reactant atom to that of a product atom,

$$V_H = k_h |\mathbf{r}_R - \mathbf{r}_P|^2.$$

When  $R$  and  $P$  are bound to  $X$  by ordinary harmonic bonds, the modified potential function,  $V'_\lambda$ , is

$$V'_\lambda = V_\lambda + V_H(\mathbf{r}_R - \mathbf{r}_P).$$

We group the terms that are exclusively  $\mathbf{r}_p$  dependent at  $\lambda = 0$

$$A'_\lambda = -k_B T \ln \frac{1}{S} \int e^{-\beta(V_X + V_R - \lambda V_R)} \times [e^{-\beta(\lambda V_P + V_H)} d\mathbf{r}_P] d\mathbf{r}_X d\mathbf{r}_R$$

because

$$\lim_{\lambda \rightarrow 0} \int e^{-\beta(\lambda V_P + V_H)} d\mathbf{r}_P = \left( \frac{\pi k_B T}{k_H} \right)^{3N_P/2},$$

where  $k_B T/k_H$  is in square angstroms ( $\text{\AA}^2$ ). Hence, the free energy converges in the limit  $\lambda \rightarrow 0$  to

$$\lim_{\lambda \rightarrow 0} A'_\lambda = -k_B T \ln \frac{1}{S_P} \left( \frac{\pi k_B T}{k_H} \right)^{3N_P/2} - k_B T \ln \frac{1}{S_X S_R} \int e^{-\beta(V_X + V_R)} d\mathbf{r}_X d\mathbf{r}_R.$$

For the limit  $\lambda \rightarrow 1$  we obtain

$$\lim_{\lambda \rightarrow 1} A'_\lambda = -k_B T \ln \frac{1}{S_R} \left( \frac{\pi k_B T}{k_H} \right)^{3N_R/2} - k_B T \ln \frac{1}{S_X S_P} \int e^{-\beta(V_X + V_P)} d\mathbf{r}_X d\mathbf{r}_P.$$

Because the restraint is applied to one atom of  $R$  and one atom of  $P$ , it follows that  $N_R = N_P$  and  $S_R = S_P$ , and that the  $k_H$ -dependent terms cancel in the difference. The overall free energy change is therefore  $k_H$  independent and given by

$$\Delta A_{0 \rightarrow 1} = A_1 - A_0 = -k_B T \ln \frac{\int e^{-\beta(V_X + V_P)} d\mathbf{r}_X d\mathbf{r}_P}{\int e^{-\beta(V_X + V_R)} d\mathbf{r}_X d\mathbf{r}_R}.$$

When the bond between  $\mathbf{r}_X$  and  $\mathbf{r}_P$  is SHAKED<sup>5</sup> (i.e., constrained to a fixed bond length), we scale  $V_H$  by  $\lambda$  and treat the SHAKED bond as a special constraint of the form

$$V_S = k_S(|\mathbf{r}_X - \mathbf{r}_P| - \mathbf{a})^2,$$

in which  $k_S \rightarrow \infty$  and  $\mathbf{a}$  is the constraint difference. The modified potential function is

$$V'_\lambda = V_\lambda + \lambda V_H(\mathbf{r}_R - \mathbf{r}_P) + V_S(\mathbf{r}_X - \mathbf{r}_P).$$

We again group the terms that are exclusively  $\mathbf{r}_p$  dependent,

$$A'_\lambda = -k_B T \ln \frac{1}{S} \int e^{-\beta(V_X + V_R - \lambda V_R)} \times [e^{-\beta(\lambda V_P + \lambda V_H + V_S)} d\mathbf{r}_P] d\mathbf{r}_X d\mathbf{r}_R,$$

because

$$\lim_{\lambda \rightarrow 0} \lim_{k_S \rightarrow \infty} \int e^{-\beta(\lambda V_P + \lambda V_H + V_S)} d\mathbf{r}_P = \int \delta(|\mathbf{r}_X - \mathbf{r}_P| - \mathbf{a}) d\mathbf{r}_P = 4\pi a^2.$$

The use of SHAKE results in a reduction of dimensionality because the locus of points that may be occupied by  $P$  is a spherical shell of radius  $\mathbf{a}$  around  $X$ . This gives rise to the  $\delta$  function, and the units of the limit are square angstroms ( $\text{\AA}^2$ ); i.e., the surface area of the spherical shell. Hence, the free energy again converges in the limit  $\lambda \rightarrow 0$  to

$$\lim_{\lambda \rightarrow 0} A'_\lambda = -k_B T \ln \frac{4\pi a^2}{S_P^{2/3}} - k_B T \ln \frac{1}{S_X S_R} \int e^{-\beta(V_X + V_R)} d\mathbf{r}_X d\mathbf{r}_R.$$

For the limit  $\lambda \rightarrow 1$  we obtain

$$\lim_{\lambda \rightarrow 1} A'_\lambda = -k_B T \ln \frac{4\pi a^2}{S_P^{2/3}} - k_B T \ln \frac{1}{S_R} \left( \frac{\pi k_B T}{k_H} \right)^{3N_R/2} - k_B T \ln \frac{1}{S_X S_P} \int e^{-\beta(V_X + V_P)} d\mathbf{r}_X d\mathbf{r}_P.$$

Thus, the overall free energy change for one path on a thermodynamic cycle is

$$\Delta A_{0 \rightarrow 1} = A_1 - A_0 = -k_B T \ln \frac{1}{S_R} \left( \frac{\pi k_B T}{k_H} \right)^{3N_R/2} - k_B T \ln \frac{\int e^{-\beta(V_X + V_P)} d\mathbf{r}_X d\mathbf{r}_P}{\int e^{-\beta(V_X + V_R)} d\mathbf{r}_X d\mathbf{r}_R},$$

and the  $k_H$ -dependent term is a constant that cancels in the difference between corresponding paths, (i.e.,  $\Delta\Delta A$ ) (Fig. 1).

It should be noted that the constant terms and final integrals are all independent of  $\lambda$  and hence independent of the path by which  $\lambda$  is changed. Thus, the states  $\lambda = 0$  and  $\lambda = 1$  are unambiguously defined, bias due to the harmonic potential is eliminated, and the final result pertains to an unbiased ensemble.

## Methods

To demonstrate this approach, we applied it to the simple conversion of methanol to methyl chloride in water. Simulations were performed using a locally modified version of CHARMM22g2,<sup>6</sup> and all-hydrogen models of methanol ( $\lambda = 0$ ) and methyl chloride ( $\lambda = 1$ ) in water. The methyl carbon and its three hydrogens ( $X$ ) were common to both models, while the —OH (the reactant,  $R$ ) and —Cl (the product,  $P$ ) atoms were separate and noninteracting when  $k_H = 0$ . The O and Cl atoms were harmonically restrained to each other's position when  $k_H > 0$ . A 14 Å stochastic boundary was centered on the methyl carbon and filled with TIP3 water as detailed elsewhere.<sup>7,8</sup> The system was equilibrated at 300 K with 8 Å nonbond cutoffs and a step size of 0.001 ps for 250 ps in each window,  $\lambda^- < \lambda < \lambda^+$ . Data was then col-

lected for 15 intervals of 10 ps each, and

$$\Delta A_{\lambda^- \rightarrow \lambda^+} = -k_B T \times \ln \left( \frac{\langle e^{-\beta \lambda^+ (V_P - V_R)} \rangle_{\lambda^-}}{\langle e^{-\beta \lambda^- (V_P - V_R)} \rangle_{\lambda^-}} \right)$$

was calculated for each interval (i.e., double-wide sampling<sup>9</sup>). The values reported for each window are the mean and standard error of the mean ( $SE = \sqrt{\sigma^2/n}$ ) for these  $n = 15$  intervals in units of kilocalories/mole (kcal/mol).

## Results and Discussion

Because  $k_H$  is found only in constant terms that should exactly cancel, free energy differences calculated using this approach should be unaffected by the use of a harmonic restraining potential. In addition, the results should be independent of the particular value assigned to  $k_H$ . Finally, any salutary effect on convergence should be most evident in windows that include values of  $\lambda = 0$  or 1.

These properties are evident in the data of Table I. Use of the modified potential changes the values obtained for individual windows (as expected), but the overall result of calculations that employ the modified potential are, within statistical uncertainty, unchanged and independent of the value assigned to  $k_H$ . There is no statistically significant difference in the overall result when using  $k_H$  values of 1, 20, or 100 kcal/mol/Å<sup>2</sup>; yet there is a

**TABLE I.**  
Free Energy Perturbation Results for the Conversion of Methanol to Methyl Chloride.

Window			$k_H = 0$		$k_H = 1$		$k_H = 20$		$k_H = 100$	
$\lambda^-$	$\lambda$	$\lambda^+$	$\Delta A$	SE	$\Delta A$	SE	$\Delta A$	SE	$\Delta A$	SE
0.995	0.999	1.000	-1.760	(0.085)	-0.695	(0.019)	-0.383	(0.004)	-0.365	(0.004)
0.98	0.99	0.995	-1.279	(0.039)	-0.829	(0.022)	-0.812	(0.010)	-0.867	(0.012)
0.93	0.96	0.98	-1.603	(0.040)	-1.139	(0.016)	-1.382	(0.015)	-1.961	(0.012)
0.85	0.90	0.93	-1.031	(0.017)	-0.952	(0.015)	-1.016	(0.016)	-1.913	(0.024)
0.60	0.75	0.85	-1.025	(0.038)	-0.854	(0.030)	-0.852	(0.031)	-2.005	(0.039)
0.40	0.50	0.60	0.522	(0.016)	0.559	(0.018)	0.629	(0.020)	0.808	(0.021)
0.15	0.25	0.40	1.188	(0.023)	1.242	(0.020)	1.371	(0.019)	2.049	(0.030)
0.07	0.10	0.15	3.418	(0.040)	3.397	(0.039)	3.769	(0.044)	5.366	(0.058)
0.02	0.04	0.07	2.426	(0.073)	2.109	(0.022)	2.264	(0.015)	2.550	(0.016)
0.005	0.01	0.02	1.881	(0.042)	1.196	(0.016)	0.935	(0.006)	0.922	(0.006)
0.000	0.001	0.005	1.997	(0.146)	0.860	(0.032)	0.363	(0.002)	0.330	(0.002)
Total	$\Delta A_{0 \rightarrow 1}$		4.735	(0.207)	4.895	(0.079)	4.895	(0.067)	4.915	(0.086)

**TABLE II.**  
**Free Energy Perturbation Results for the Conversion of a Peptide —NH— to —O— in Reference 10.**

Window			$k_H = 0$		$k_H = 20$	
$\lambda^-$	$\lambda$	$\lambda^+$	$\Delta\Delta A$	SE	$\Delta\Delta A$	SE
0.00	0.01	0.02	0.500	0.49	0.076	0.014
0.02	0.03	0.04	0.075	0.04	0.061	0.005
0.04	0.05	0.07	0.103	0.04	0.193	0.010
0.07	0.10	0.15	0.174	0.06	0.459	0.020
0.15	0.20	0.25	0.348	0.04	0.671	0.030
0.25	0.30	0.35	0.448	0.05	0.688	0.018
0.35	0.40	0.45	0.431	0.05	0.645	0.021
0.45	0.50	0.55	0.526	0.03	0.585	0.020
0.55	0.60	0.65	0.428	0.07	0.413	0.029
0.65	0.70	0.75	0.815	0.09	0.648	0.029
0.75	0.80	0.85	0.410	0.10	0.010	0.019
0.85	0.90	0.93	0.125	0.12	0.183	0.033
0.93	0.95	0.96	0.186	0.04	0.073	0.020
0.96	0.97	0.98	0.071	0.07	0.128	0.021
0.98	0.99	1.00	0.042	0.28	0.063	0.023
Total	$\Delta\Delta A_{0.00 \rightarrow 1.00}$		4.7	0.6	4.90	0.09

clear improvement in the overall convergence of the calculation. Improved convergence is most evident in windows near the extreme values of  $\lambda$ . Indeed, most of the imprecision in the  $k_H = 0$  calculation is contributed by a single window ( $0.000 \leq \lambda \leq 0.005$ ), and a force constant of  $k_H = 20$  reduces the imprecision of this window to a negligible level.

We applied this approach to a variety of other systems and had typically even better improvement in convergence. For example, in an extension of work published earlier,<sup>8</sup> the relative free energy of acetyl-D-alanyl-D-alanine versus acetyl-D-alanyl-D-lactate binding to a glycopeptide antibiotic was investigated. This conversion involved the substitution of —O— (the reactant, *R*) for —NH— (the product, *P*) in the dipeptide backbone. We found the free energy difference between these two ligands to be  $4.7 \pm 0.6$  kcal/mol using a conventional approach and  $4.90 \pm 0.09$  kcal/mol using a harmonic restraint with  $k_H = 20$  and a dynamics trajectory of only half as long<sup>10</sup> (Table II). Clearly, this degree of improved convergence and precision is of tremendous value for rational drug design efforts.

When applying a harmonic restraint to the annihilation of water in a protein cavity, it has been suggested that the optimal value for  $k_H$  is the value that best matches the mean square fluctuations of the water in a fully interacting simulation.<sup>4</sup> In this case, however, the optimal choice is not

clear because the restrained atoms are both bonded to a common atom and hence their motions relative to each other are highly anisotropic. Increasing the value of  $k_H$  leads to greater improvement in convergence for windows at or near the extremes of  $\lambda$ , but larger values of  $k_H$  do not necessarily improve *overall* convergence. This is due to a detrimental affect on convergence for midrange values of  $\lambda$  (see Table II), presumably as a consequence of bond-length distortions induced when bonds between X—R and X—P are of unequal length and the magnitude of the force constant approaches the magnitude of the force constants applied to the bonds. The force constant has a practical maximum in that the oscillation frequency of the restrained atoms must be slow compared to the simulation time step. We found that  $k_H = 20$  yields satisfactory results in a variety of cases.

We conclude that the application of a simple harmonic restraint in the manner described is appropriate for use when calculating free energy differences between systems of this type and that it significantly enhances the convergence behavior of the dual-topology approach.

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